The Relationship Between Prenatal Exposure to Lead and Congenital Anomalies

177755

Herbert L. Needleman, MD; Michael Rabinowitz, PhD; Alan Leviton, MD; Shai Linn, MD; Stephen Schoenbaum, MD

● We obtained umbilical cord blood from 5,183 consecutive deliveries of at least 20 weeks' gestation and analyzed them for lead concentration. Those demographic and socioeconomic variables, including lead, which were shown on univariate analysis to be associated with increased risk for congenital anomalies were evaluated and controlled by entering them into a stepwise logistic-regression model with malformation as the outcome. Coffee, alcohol, tobacco, and marijuana use, which were associated with lead level, but not risk of malformation, were also controlled. The model was reduced in steps by eliminating the variables with the highest *P* value, until the most parsimonious model was created. The relative risk for anomalies associated with lead was then calculated while holding other covariates constant. Lead was found to be associated, in a dose-related fashion, with an increased risk for minor anomalies.

(JAMA 1984;251:2956-2959)

SINCE the beginning of the 20th century, lead has been considered a potential human teratogen. British factory inspectors of that period reported an increase in the incidence of infertility, abortion, stillbirth, fetal death, and macrocephaly associated with industrial lead exposure.[1] It is surprising that for the next 50 years, little attention was given to the possible embryotoxic effects of lead. Lead crosses the placenta, and in the rat has been shown to be mobilized from maternal stores during pregnancy.[2] Studies in three rodent species have shown that lead readily produces neural tube lesions.[3]

Women who lived during pregnancy in homes where the drinking water had excessive amounts of lead, bore a significantly (*P*<.01) higher proportion of retarded infants.[4] Increased placental lead concentrations have been reported in stillbirths, and in infants with congenital anomalies.[5] Lead also affects the male gamete. Increased numbers of chromosomal alterations have been reported in lead workers, and abnormalities in sperm number, vigor, and morphologic features have been demonstrated in both experimental animals and in workers.[6]

We and others have shown a deleterious effect of exposure to lead at low dose on the neuropsychological function of school-age children.[7] Because younger, developing organisms may be most vulnerable to lead, the study of the effects of exposure on the human fetus is a matter of considerable interest. No epidemiologic studies of umbilical cord blood lead levels and their relationship to neonatal outcome have been reported.

To evaluate lead's impact during pregnancy, we measured the concentration of lead in umbilical cord blood in a large cohort of neonates from a single obstetrical hospital, and examined its relationship to a number of outcomes measured at birth, including congenital anomalies.

METHODS
Study Sample

Between April 1979 and March 1980, at the Boston Hospital for Women, 5,183 mothers delivered live-born neonates of at least 20 weeks' gestational age. In the delivery room, during the fourth stage of labor, 5-mL samples of umbilical cord blood were collected in metal-free tubes from 97% of the neonates. Mothers were interviewed by trained personnel on the first or second postnatal day, employing a structured questionnaire previously used in other studies of reproductive outcome.[1] In addition to standard demographic variables, the questionnaire focused on reproductive and medical history and on exposure to medicines, alcohol, tobacco, coffee, tea, and marijuana.

Permission for the interview was sought from 84% of the eligible mothers. Reasons for not contacting the mother were lack of interviewers (14%) and lack of consent of obstetrician (1.5%). Of those mothers approached, 90% were interviewed. Reasons for not being interviewed were early discharge (5%), refusal (3%), language barrier (1.6%), and maternal medical condition (0.1%).

From hospital records, data on each newborn's status, the pregnancy, and delivery were obtained. A small number of records (1.2%) could not be found after several systematic searches. These cases were excluded from the study.

Malformations were classified from the physicians' notes in the charts by employing the coding scheme developed by the Centers for Disease Control's Malformation Surveillance Program. The newborn examinations were done by pediatric house officers assigned to the neonatology service. Whenever a malformation could not be classified by this scheme, we used the criteria employed in the Collaborative Perinatal Project.

Lead determinations were obtained for 97% of the births during the period of the study. They were analyzed in duplicate by an anodic-stripping voltammetry to a precision of 2 mg/L as previously reported.[8]

Statistical Analysis

Combined lead and maternal interview data were available for 4,354 births. Neonates were classified according to their blood lead levels into quartiles. To identify those maternal and neonate variables that were associated with lead level, we cross tabulated the variables with lead and evaluated whether the overall distribution of demographic, pregnancy, and delivery events differed across lead groups by χ^2. The univariate relationship between umbilical cord blood lead level and certain outcomes was then evaluated by testing for linear trends.

Logistic regression analysis was then used to determine the contribution of lead

to risk of malformation while controlling for the effect of other potentially confounding variables. Those variables that previous univariate analyses had shown to be associated with anomalies at $P<.3$, were entered into the logistic regression model. These potentially confounding variables were gestational age, birth weight, history of either spontaneous or induced abortion, maternal parity, and age. Initially all variables and their interactions with lead were entered and the fitness of the model quantified in terms of its log likelihood. Continuous variables were not degraded. Lead was logarithmically transformed (base 10) to normalize its distribution. Maternal age was entered both as a linear and quadratic term. In sequential steps, the variable whose coefficient had the highest P value was dropped from the model. Priority was given to eliminating interaction terms. After each step a new model was recalculated. The new model was retained only if Wilk's criterion was met, ie, the change in the model's log likelihood was not significantly degraded ($P>.05$) by dropping a variable. This step-down iteration generated a set of increasingly parsimonious models. Each model was also calculated without including the term for lead to see if that step met Wilk's criterion for additional predictive information. Because this procedure excludes a subject if any single observation is missing, a minimum data set with complete interview data was required to count a subject. This limitation reduced the number of subjects by only 0.8%.

Coffee, alcohol, tobacco, and marijuana use, which were associated with lead level but not with malformation risk, were included in the initial model. Entering these variables, categorized either according to their associated risk of malformation or as continuous values, did not increase the predictability of the model.

The covariate-adjusted relative risk of malformations associated with lead level was then calculated while maintaining the other predictive variables constant." As is customary with probit analysis, we assumed an exponential relationship between lead's β coefficient from the logistic model and the observed malformation rates.

RESULTS

Women whose neonate's cord blood lead levels were elevated tended to be divorced, unmarried, on public assistance, to consume more alcohol, coffee, and tobacco, and to have had an induced abortion (Table 1). Decreased lead levels were associated with being white, Jewish, college educated, and having had full-term pregnancies in the past. Maternal characteristics not

significantly related to lead included aspirin or acetaminophen use, venereal disease, diabetes or hypertension, maternal occupation, age of menarche, or contraceptive use. Bleeding during the first trimester and premature labor were more frequent in women with lower levels of lead (Table 2). In general, perinatal blood lead levels were not altered by late pregnancy events, presentation, or mode of delivery.

Low birth weight, short gestation, low Apgar score, jaundice, blood type,

and neonate gender were unrelated to lead. The occurrence of respiratory distress varied inversely with lead level.

The incidence of minor malformations was associated with cord blood lead level (Table 3). Multiple or major malformations did not show this pattern. The commonest anomalies found were hemangiomas and lymphangiomas (14/1,000 births), hydrocele (27.6/1,000 males), minor skin anomalies such as skin tags and papillae (12.2/1,000 births), and undescended

Table 1.—Number of Mothers With Selected Demographic and Medical Characteristics Within Each Cord Blood Lead Category

| Characteristic | Lead Category, µg/dL | | | |
	Low 0 - ≤4.8	Mid-Low 4.9 - ≤6.8	Mid-High 6.8 - ≤8.8	High 8.7 - ≤38.1
Total No.	1,118	1,068	1,105	1,063
Demographic				
Age ≥35 yr	97	96	109	115
Age ≤18 yr	17	17	24	24
On welfare	139	176	184	237*
White	838	797	790	724†
Jewish	149	119	113	83*
College educated	772	691	684	599*
Divorced or cohabitating	22	46	54	56*
Habits				
Alcohol, third trimester, ≥7/wk	18	18	28	49*
Coffee, ≥3/day	72	84	118	134*
Tobacco, third trimester, ≥3/day	147	189	234	303*
History				
Gravidity >1	728	685	694	659
Parity >1	585	546	539	486†
Previous stillbirth(s)	26	29	30	30
Induced abortion(s)	168	189	202	220†
Miscarriage(s)	187	187	194	187
Ponderal index‡				
<18, thin	82	52	70	68
>30, obese	36	33	46	49

*$P<.01$, x^2.   †$P<.05$, x^2.   ‡Weight (kg)/height^2 (m).

Table 2.—Number of Selected Pregnancy Events and Delivery Characteristics Within Each Lead Category

| Characteristic | Lead Category | | | |
	Low	Mid-Low	Mid-High	High
Pregnancy events				
Bleeding in				
1st trimester	104	91	99	77*
2nd trimester	22	29	32	23
3rd trimester	29	34	36	32
Toxemia or eclampsia	39	33	37	48
Prepartum admissions	87	106	123	90†
Premature labor	44	37	32	28*
Delivery characteristics				
Breech presentation	38	47	47	46
Placenta previa	3	8	5	3
Premature rupture of membrane	56	47	50	38
Placenta abruptio	5	9	5	7
Fetal distress	40	34	34	40

*$P<.01$, x^2.   †$P<.05$, x^2.

Table 3.—Number of Infants With Selected Characteristics Grouped by Lead Category

Characteristic	Lead Category			
	Low	Mid-Low	Mid-High	High
Birth weight <2,500 g	84	76	86	83
Gestation <37 wk	66	73	76	76
Malformations				
Any	86	72	101	102*
≥2	9	8	17	11
Major	32	25	32	31
Minor	54	47	69	71*
Hydrocele or undescended testicle	21	11	23	27
1 minute Apgar <8	74	60	76	66
Respiratory distress	72	57	57	47*
Neonatal jaundice	290	236	284	261

*Significant (P<.05) linear trends across lead categories.

Table 4.—Logistic Models Predicting Malformations*

Variable	$\beta, \pm SE$	
	With Lead	Without Lead
Logistic Model		
Log_{10} lead, mg/L	0.66±.27	...
Maternal age, yr	−.014±.010	−.013±.010
Birth weight, kg	.0036±.0021	.0036±.0021
Gestational age, wk	−.040±.025	−.037±.024
Race, B	0.31±.15	.33±.15
Agreement of Model Fit to Data		
−2 log likelihood	2,457.25	2,463.14
Model χ^2	17.61	11.71
df	5	4
P	.004	.020

*Maternal age, gestational age, birth weight, race, and blood lead are the significant predictors in this most terse model, given with and without blood lead terms. Information about lead significantly (P=.015) improves the model's predictive power.

Table 5.—Covariate-Adjusted Relative Risk of Malformation at Selected Blood Lead Levels

Blood Lead, µg/dL	Relative Risk*	% of Neonates at Greater Lead Levels
0.7	1.0	98.7
6.3	1.87 (1.44-2.42)	50.0
15	2.39 (1.66-3.43)	1.7
24	2.73 (1.80-4.16)	0.2

*Mean ± 95% confidence interval, β=0.655±0.273 (SE).

testicles (11/1,000 males). No particular type of malformation was associated with lead.

The observed associations of malformations and lead with parity and past abortion, as well as the interactions of lead with other personal factors, required multivariate analysis to quantify the contribution of each variable to the risk of malformation. A variety of logistic regression analyses included different combinations of variables; increasingly terse models were achieved, both with and without lead as a covariate. Information about lead levels significantly improved the powers of prediction of these models (P<.02). Adding information about alcohol, tobacco, or cof-

fee use changed the model log likelihood by only 0.5 (P>.26). Table 4 gives the most parsimonious model.

Of considerable interest are the calculations summarized in Table 5. The β coefficient for lead was determined by fitting the model with lead plus maternal age, birth weight, race, and gestational age. Holding the other factors constant, the relative risk of a child's demonstrating a malformation at birth increases by 50% as lead levels increase from 0.7 to the mean of 6.3 mg/L, and increases another 50% at 24 mg/L.

COMMENT

These data show a relationship between umbilical cord blood lead

levels and the risk of minor congenital anomaly, while controlling for other covariates. No single characteristic anatomic defect was found; the overall incidence of many different anomalies was raised in newborns with high lead burdens. This suggests that lead may interact with other teratogenic risk factors to enhance the probability of abnormal outcome. Lead was not found to be associated with decreased birth weight, shortened gestation, Apgar score, respiratory distress, or the presence of jaundice.

The anomalies discovered in this study are in themselves of little health consequence, but may be important as markers of impaired development. Marden et al[11] examined 4,412 newborns, and reported that the risk of having a major anomaly was increased in those newborns with two or more minor anomalies. They suggest that the presence of minor anomalies may be a clue to the presence of more serious malformations.

This study may be considered a "level 2" study, that is, it employed preexisting records (hospital charts) to collect data, and cross tabulated these data with blood lead levels measured in our laboratory. Diagnosis of anomaly was made by clinicians (pediatric residents assigned to the neonatology service) in the conduct of their routine neonatologic duties, and observation was limited to the first days of life. We did not examine these newborns ourselves. A potential weakness of this study is that the surveillance was not carried out by specialists in teratology. It is quite likely then that a number of birth defects were missed, and some will be diagnosed at later ages. It is not likely, however, that these misclassifications were biased with regard to their lead exposure because exposure was ascertained separately and was not known by either mother or pediatrician.

Valid comparisons of the rates of anomalies reported by different observers are difficult because of variations in diagnostic criteria and other risk factors that may vary across groups. Our reported rate for major malformations, 3%, is somewhat higher than that reported by Heinonen et al[12] (1.8%), or Christianson et al[13] (1.2%). This may be because the

Boston Hospital for Women is a referral center for high-risk pregnancies. For technical reasons, we were unable to sample the mother's blood lead levels during the three trimesters of pregnancy, and cannot, therefore, report directly on the exposure of the fetuses in early development. Sequential blood levels have been measured in another study, however, and no significant change in concentration was observed during the period of gestation.[4]

Among the mechanisms through which teratogens act are germinal and somatic mutations, interference with mitosis, chromosomal alterations, membrane changes, and disturbances in nutrition or energy sources. Lead has been shown to act on many of these mechanisms. Lead can affect the fidelity of DNA synthesis in vitro,[3] and in vivo has been shown to perturb cell proliferation and DNA synthesis.[4] Lead has recently been shown to cleave the sugar-phosphate backbone of transfer RNA in catalytic fashion.[17] Mammalian cells exposed to lead in vitro have shown increases in dicentric chromosomes and defects in centralization.[18]

Lead inteferes with embryonic nutrition and energy supply at a number of sites. It competes with other cations such as zinc, iron, or calcium, and thereby limits their availability

at critical sites.[19] Lead interferes with mitochondrial function,[20] depresses the synthesis of cytochromes, and thus alters the energy supply to a number of organs. Delayed appearance of cytochromes in developing rat brains have been reported at low tissue levels of lead. This has been paralleled by delayed synaptic development.[20,21]

Lead administered to mammals reliably produces fetal death, abortion, and retarded fetal and postnatal growth, CNS hemorrhage, and hydrocephalus.[22] Lead given at lower doses during neurogenesis produces no macroscopic alterations in structure, but inteferes with cortical connectivity as measured by synaptic number and maturity.[23] Behavioral studies of the offspring of mother and father rats exposed to lead before conception showed impaired learning,[24] suggesting that the gametoxic effects of lead can be expressed in behavioral deficit at doses well below those which produce macroscopic anatomic abnormality.

In this study we restricted our inquiry to the identification of growth retardation and structural abnormalities visible to the clinician during the first few days of the newborn's life. We were able to evaluate the contribution of a number of covariates, including coffee, alcohol,

and tobacco use. These variables did not increase the predictive power of lead when added to the model. Indicators of nutritional status such as protein, zinc, and, calcium intake were not evaluated in this investigation. Because deficiency of these factors may affect outcome and be associated with increased lead absorption, they may represent potential confounders. The study was not designed to measure the impact of lead on fetal death, abortion, or other embryotoxic outcomes such as impaired postnatal growth or CNS development.

Whether children in this birth cohort found to be without anomaly will later demonstrate malformations, growth, or behavioral deficits remains to be determined. We are currently following a sample of these children in the high, middle, and low range of cord blood lead levels at regular intervals and evaluating both their physical and neuropsychological development.

References

1. Oliver T: A lecture on lead poisoning and the race. Br Med J 1911;1:1096-1098.

2. Buchet JP, Lauwerys R, Roels H, et al: Mobilization of lead during pregnancy in rats. Int Arch Occup Environ Health 1977;40:33-36.

3. Gerber GB, Leonard A, Jacquet P: Toxicity, mutagenicity and teratogenicity of lead. Mutat Res 1977;76:115-141.

4. Beattie AD, Moore MR, Goldberg A, et al: Role of chronic low level lead exposure in the etiology of mental retardation. Lancet 1975; 1.589-592.

5. Wibberly DG, Khera AK, Edwards JH, et al: Lead levels in human placentas from normal and malformed births. J Med Genet 1977;14:339-345.

6. Deknudt GH, Leonard A, Ivanov B: Chromosome aberrations observed in male workers occupationally exposed to lead. Environ Physiol Biochem 1973;3:132-138.

7. Needleman HL, Gunnoe CG, Leviton A, et al: Deficits in psychologic performance and classroom behavior in children with elevated dentine lead levels. N Engl J Med 1979;300:689-695.

8. Linn S, Schoenbaum SC, Monson RR, et al: No association between coffee consumption and adverse outcomes of pregnancy. N Engl J Med 1982;306:141-145.

9. Rabinowitz MR, Needleman HL: Temporal trends in the lead concentration of umbilical cord blood. Science 1982;216:1429-1431.

10. Kleinbaum DG, Kupper LL, Morganstern H: Epidemiologic Research: Principles and Quantitative Methods. Wadsworth Publishing Co, Belmont, Calif, 1982, p 422.

11. Marden PM, Smith DW, McDonald MJ: Congenital anomalies in the newborn infant, including minor variations. J Pediatr 1964; 62:357-371.

12. Heinonen OP, Slone D, Shapiro S: Birth Defects and Drugs in Pregnancy. Boston, John Wright-PSG, 1982, p 425.

13. Christianson R, Van den Berg B, Milkovick L, et al: Incidence of congenital anomalies among white and black live births with long term follow up. Am J Public Health 1981; 71:1333-1341.

14. Lubin AH, Caffo AL, Reece R: A longitudinal study of interaction between environmental lead and blood lead concentrations during pregnancy, at delivery, and the first 6 months of life. Pediatr Res 1978;12:425.

15. Sirover MA, Loeb LA: Infidelity of DNA synthesis in vitro: Screening for potential mutagens or carcinogens. Science 1976;194:1434-1436.

16. Choie DD, Richter G: Stimulation of DNA synthesis in rat kidney by repeated administration of lead. Proc Soc Exp Biol Med 1972; 142:446-449.

17. Brown RS, Hingerty BE, Dewan JC, et al: Pb(II)-catalysed cleavage of the sugar-phosphate backbone of yeast tRNA-implications for

lead toxicity and self-splicing RNA. Nature 1983;303:543-546.

18. Obe G, Beek B, Dudin D: Some experiments on the action of lead acetate on human leucocytes in vitro. Mutat Res 1975;29:283.

19. Mahaffey K, Michaelson A: Interactions between lead and nutrition, in Needleman HL (ed): Low Level Lead Exposure: The Clinical Implications of Current Research. New York, Raven Press, 1980, pp 159-200.

20. Holtzman J, Hsu JS: Early effects of lead on immature rat brain mitochondrial respiration. Pediatr Res 1976;10:70-75.

21. McCauley PT, Bull RJ, Lutkenhoff SD: Association of alterations in energy metabolism with lead-induced delays in rat cerebral cortical development. Neuropharmacology 1979;18:93-101.

22. McClain RM, Becker BA: Teratogenicity, fetal toxicity, and placental transfer of lead nitrate in rats. Toxicol Appl Pharmacol 1975; 31:72-82.

23. Averill DA, Needleman HL: Neonatal lead exposure retards cortical synaptogenesis in the rat, in Needleman HL (ed): Low Level Lead Exposure: The Clinical Implications of Current Research. New York, Raven Press, 1980, pp 201-210.

24. Brady K, Herrera Y, Zenick H: Influence of parental lead exposure on subsequent learning ability of offspring. Pharmacol Biochem Behav 1975;3:561-565.